Autogenous Regulation of the Positive Regulatory *qa-1F* Gene in *Neurospora crassa*

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In *Neurospora crassa*, the *qa-1F* regulatory gene positively controls transcription of all genes in the quinic acid (*qa*) gene cluster. *qa-1F* is transcribed at a low, uninduced level but is subject to strong (50-fold), autogenous regulation as well as to control by the negative regulatory gene, *qa-1S*, and the inducer quinic acid. Cloned *qa-1F* DNA sequences hybridize to two related mRNAs of 2.9 and 3.0 kilobases. When wild-type (*qa-1F*⁺) cultures are transferred to inducing conditions, *qa-1F* mRNA increases for 4 h, remains somewhat level, and decreases after 8 to 10 h. That this control is autogenous, i.e., that the *qa-1F* gene controls the synthesis of its own mRNA, is indicated by the presence of approximately the same low level of *qa-1F* mRNA in poly(A)⁺ RNA from noninducible *qa-1F*⁻ mutant cultures under inducing conditions as that observed in uninduced wild-type cultures. The *qa-1S* gene also regulates the transcription of *qa-1F*, since a *qa-1S*⁻ mutant, whether in noninducing or inducing conditions, contains a level of *qa-1F* mRNA that corresponds to the low level observed in uninduced wild-type cultures. These results corroborate the hypothesis (M. E. Case and N. H. Giles, Proc. Natl. Acad. Sci. USA 72:553-557, 1975; V. B. Patel, M. Schweizer, C. C. Dykstra, S. R. Kushner, and N. H. Giles, Proc. Natl. Acad. Sci. USA 78:5783-5787, 1981; L. Huiet, Proc. Natl. Acad. Sci. USA 81:1174-1178, 1984) that the *qa-1F* gene encodes an activator protein and acts positively in controlling transcription of itself and the other *qa* genes.

The quinic acid (*qa*) gene cluster of *Neurospora crassa*, a well-defined system for studying eucaryotic gene regulation, comprises seven tightly linked genes that enable *N. crassa* to utilize quinic acid as a carbon source (11, 12). The two *qa* regulatory genes in this cluster, *qa-1S* and *qa-1F*, act together with the inducer quinic acid to control the expression of each other and of the five structural genes (5, 15, 22). Three of the five structural genes, *qa-2*, *qa-3*, and *qa-4*, encode three different inducible enzymes catalyzing the first three reactions in the quinic acid catabolic pathway (11). Transcripts from the other two presumptive structural genes, *qa-x* and *qa-y*, are induced by quinic acid, but the functions of these genes are unknown (22). Based on genetic (5) and molecular evidence (15), the proposal has been made that the *qa-1S* and *qa-1F* genes encode a repressor and an activator protein, respectively. In uninduced wild type, transcription of *qa-1S* occurs at a very low level but is induced by quinic acid. This induction requires products of both the *qa-1S* and *qa-1F* genes (15). In this study, by utilizing mutants in each of these two genes, we have attempted to determine the roles of both regulatory genes in controlling the synthesis of *qa-1F* mRNA. We have found that, in wild type, the *qa-1F* gene is transcribed at a low, uninduced level but is subject to strong (50-fold), autogenous regulation during induction by quinic acid as well as to control by the negative regulatory gene, *qa-1S*. Thus, we consider these results to corroborate the hypothesis (5, 15) that, by encoding an activator protein, the *qa-1F* gene acts positively in controlling transcription of itself and the other *qa* genes.

MATERIALS AND METHODS

Strains. The *qa-1F*⁻ (M158 and M162), *qa-1S*⁻ (M141), and *qa-1S*^{ts} (M105) mutants were originally isolated by Howard

Rines (Genetics 74:s230, 1973) in an *arom-9*⁻ mutant (M6-11) in the wild-type 74A background. Since these double mutants lack both biosynthetic and catabolic dehydroquinase activities, they are unable to grow on sucrose with Fries salts without aromatic amino acids. Subsequently, M. Case crossed the double mutants *qa-1F*⁻ *arom-9*⁻ (M158), *qa-1S*⁻ *arom-9*⁻ (M141), and *qa-1S*^{ts} *arom-9* (M105) to a *qa-1F*⁺ *me-7*⁻ *arom-9*⁺ (allele no. 4894, Fungal Genetics Stock Center) strain. The *qa-1F* and *me-7* genes are tightly linked. The isolates from these crosses were selected by their ability to grow on sucrose with Fries salts without the aromatic amino acids and to be noninducible in the presence of quinic acid. The constitutive *qa-1S*^c (M105-R12-1.5) used in this study was a UV-induced revertant of the temperature-sensitive mutant M105 (*qa-1S*^{ts}) that produces a high level of catabolic dehydroquinase at 25°C, but not at 35°C, in the absence of inducer (29). The triple mutant (*qa-3*⁻ *qa-4*⁻ *arom-1*⁻; strain no. 336-3-28-20A-1183-1) lacks quinate dehydrogenase and dehydroshikimate dehydratase, which are enzymes in the quinate catabolic pathway, as well as dehydroshikimate reductase, an enzyme in the aromatic amino acid biosynthetic pathway.

Conditions for growth and enzyme induction. In general, the conditions for growth and enzyme induction in quinic acid used in this investigation have been described by Chaleff (7). Specifically, to obtain a sufficient quantity of mycelia for RNA extraction and enzyme assays, *Neurospora* mycelia were grown from conidia for 17 to 20 h in 1.5% sucrose or glucose solution with Fries salts and then transferred to experimental conditions. When strains having an *arom-9*⁻ or *arom-1*⁻ mutation were grown, all media were supplemented with aromatic amino acids.

The experimental conditions involved a noninduced control, a fresh 1.5% sucrose or glucose solution with Fries salts, and an inducing condition, 0.3% quinic acid solution with Fries salts. Cycloheximide was added to *N. crassa* cultures 15 min before harvesting to prevent polysomal

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FIG. 1. Transcriptional map of the *N. crassa qa* gene cluster and localization of DNA probes used for RNA blot hybridization. (A) Diagram indicating the transcriptional direction of the *qa* mRNAs and primarily summarizing data with respect to *qa* inducible genes (28) and *qa* regulatory genes (15). It also includes data from Patel et al. (22) and Alton et al. (1). *qa-x* and *qa-1S* contain small intervening sequences. Each *qa* gene, except *qa-y*, encodes two or more transcripts. The heterogeneity in size of the *qa-3* mRNAs and *qa-1F* mRNAs are shown (solid bars) since data on these mRNAs are specifically presented in this paper. Both *qa-1F* and *qa-3* mRNA heterogeneity occur at the 5' end (28; R. Geever et al., unpublished data). (B) Localized *Neurospora* DNA inserts (hatched bars) of nick-translated (25) plasmids used as probes for RNA blot hybridizations. pQA3 and pLH40 were kindly donated by B. Tyler and L. Huiet, respectively.

runoff (19) and improve mRNA recoveries. Specific details for growth conditions are also stated in the figure legends.

RNA preparation and gel electrophoresis. RNA was extracted from mycelia with phenol-chloroform and proteinase K as previously described (24). Poly(A)⁺ RNA was prepared by oligo(dT)-cellulose chromatography (3).

Methylmercury hydroxide was used as the denaturing agent for RNA that was fractionated by agarose gel electrophoresis (4). It should be noted that the overall lengths in kilobases of *qa* mRNA sequences reported here are shorter than those reported by glyoxalation (22). Relative sizes, however, remain the same.

RNA blotting and hybridization conditions. RNA was blotted onto diazobenzylxymethyl-paper as described by Alwine et al. (2). Before transfer from gels to diazobenzylxymethyl-paper, the quantity and quality of the various poly(A)⁺ RNAs were verified by ethidium bromide staining. In addition, all poly(A)⁺ RNA blots were hybridized to the radiolabeled histone H3 gene to use the H3 mRNA as an internal standard for the various preparations of poly(A)⁺ RNA from different strains and experimental conditions. Plasmid pNcH3H4 containing the *Neurospora* histone H3 and H4 genes was kindly donated by L. P. Woudt (Biochemisch Laboratorium, Vrije Universiteit, Amsterdam, The Netherlands). Prehybridization and hybridization conditions were the same as those described by Patel et al. (22), except that 0.1% sodium dodecyl sulfate was added (27). DNA plasmid and restriction endonuclease fragments used as probes for hybridization were labeled with ³²P either by nick translating (25) or by using the large fragment of DNA polymerase I with random oligodeoxyribonucleotides as primers, as described by Feinberg and Vogelstein (9). The amount of radiolabeled DNA probe added during hybridization was 2×10^6 to 4×10^6 cpm of ³²P as determined by Cerenkov counting per RNA sample transferred to blot. Blots were washed as described by Patel et al. (22) with two additional washes, one at 65°C for 1 h, followed by a second at room temperature for 15 min, in 0.1% sodium dodecyl sulfate–30 mM sodium chloride–3 mM sodium citrate–10 mM sodium phosphate buffer (pH 7.5). Blots were air dried and exposed to Kodak X-Omat AR (XAR-5) film with a Cronex intensifying screen at –70°C for 4 h to a week.

When RNA blots were reused for hybridizations with different probes, the radioactive probes were removed by

washing blots at 65°C in 75% formamide–0.1% sodium dodecyl sulfate–2 mM sodium EDTA–0.2% bovine serum albumin–0.02% Ficoll–0.02% polyvinylpyrrolidone–10 mM sodium phosphate buffer (pH 6.5) for 1 h. Then, before subsequent prehybridization, blots were washed at 42°C in 30 mM sodium chloride–3 mM sodium citrate–0.1% sodium dodecyl sulfate–2 mM sodium EDTA–10 mM sodium phosphate buffer (pH 6.5) for at least 1 h.

Quantitation of *qa-1F* mRNA and *qa-3* mRNA. The levels of *qa-1F* mRNA and *qa-3* mRNA in uninduced and induced wild-type *Neurospora* cultures were initially quantitated by two methods. The first was by densitometric scanning of autoradiograms of an RNA blot hybridized to specific ³²P-labeled DNA probes with a Beckman DU-8 spectrophotometer. The second was by counting the radioactivity of the excised bands hybridized to the specific ³²P-labeled probes with the same blot as in the first method. Both methods gave similar results; therefore, subsequent RNA blots were quantitated only by the first method. *qa-3* mRNA in uninduced cultures was only detectable by counting the radioactivity of the excised bands or by exposing X-ray film for a month to the hybridized RNA blot.

Calculation of the number of *qa-1F* mRNA molecules per nucleus. We calculated the approximate number of *qa-1F* mRNA molecules per nucleus in induced wild-type cultures based on the following assumptions. The *Neurospora* genome is 2.7×10^7 base pairs. Ninety percent (2.43×10^7 base pairs) are single-copy sequences which have the potential for 18,000 different genes (17). Therefore, the average size mRNA is 1,350 bases. Based on the RNA/DNA ratio and taking into account the level of rRNA, there are approximately 10,000 to 100,000 mRNA molecules per nucleus. From our DNA-RNA blot analyses, we have determined that *qa-1F* mRNA (2,900 bases) is 0.002% of poly(A)⁺ RNA from induced wild-type cultures. If the number of mRNA molecules ranges from 10,000 to 100,000, the range of *qa-1F* mRNA is from 0.1 to 1 molecule per nucleus.

RESULTS

Figure 1A shows the present transcriptional map of the *qa* cluster. In the present study, DNA from recombinant plasmid pLH40, which contains the smallest *N. crassa* insert (3.3 kilobases; Fig. 1B) capable of transforming recipients of

FIG. 2. Levels of *qa-1F* mRNA and *qa-3* mRNA in poly(A)⁺ RNA from *qa-1F*⁺ wild-type and *qa-1F*⁻ mutant *N. crassa* cultures. In panel A, *qa-1F* mRNA is detected in the various poly(A)⁺ RNAs by blot hybridization with pLH40 (Fig. 1B). The lanes contain the following poly(A)⁺ RNAs: a, induced wild type, 5 μ g (this is a shorter exposure of lane e); b, uninduced wild type, 20 μ g; c, wild type at 0 h of induction, 5 μ g; d, wild type at 2 h of induction, 5 μ g; e, wild type at 4 h of induction, 5 μ g; f, wild type at 8 h of induction, 5 μ g; g, wild type at 10 h of induction, 5 μ g; (in lanes h through m, all cultures were transferred to either sucrose (SUC), glucose (GLU), or quinic acid (QA) for 4 h) h, a *qa-1F*⁻ mutant (M158) in sucrose, 20 μ g; i, M158 in quinic acid, 20 μ g; j, an *arom-9*⁻ mutant in sucrose, 10 μ g; k, an *arom-1*⁻ *qa-3*⁻ *qa-4*⁻ triple mutant in glucose, 5 μ g (the level of *qa-1F* in this sample was compared densitometrically with that of the control, uninduced wild type in glucose); l, M158 *arom-9*⁻ double mutant in sucrose, 20 μ g; m, *qa-1F*⁻ M162 *arom-9*⁻ double mutant in sucrose, 20 μ g. (The *qa-1F* mRNA of mutant 162 is 1.9 versus 2.9 kilobases for wild-type and mutant 158.) Poly(A)⁺ RNAs from wild-type (74A) cultures (lanes a through g) are from the same experiment; poly(A)⁺ RNAs from the mutant cultures are from different experiments. In all experiments, however, the level of *qa-1F* mRNA was compared densitometrically with the control uninduced (sucrose or glucose) sample for each respective DNA-RNA blot. In panel B, *qa-3* mRNA is indicated. The lanes contain the same poly(A)⁺ RNAs as in panel A because the blot that was used in panel A was washed (procedure described in Materials and Methods) and subsequently hybridized to probe pQA3 (Fig. 1B).

the noninducible *qa-1F*⁻ type only (15), has been used as a probe to examine regulation of the *qa-1F* gene at the level of transcription. Mycelia for poly(A)⁺ RNA extraction and enzyme assays were grown from conidia for 17 to 20 h in 1.5% sucrose or glucose solution with Fries salts and then transferred either to the control noninducing condition, fresh 1.5% sucrose or glucose with Fries salts, or to the inducing condition, 0.3% quinic acid with Fries salts.

The quality and quantity of the various poly(A)⁺ RNAs were verified by ethidium bromide staining and by hybridization of all RNA blots to the radiolabeled *Neurospora* histone H3 gene. The levels of *qa-1F* and *qa-3* mRNAs were compared with that of histone H3 mRNA to standardize the various poly(A)⁺ RNA preparations.

Autogenous regulation of the *qa-1F* gene. Cloned *qa-1F* DNA sequences (pLH40) hybridize to two related mRNAs of 2.9 and 3.0 kilobases in induced wild-type poly(A)⁺ RNA (Fig. 2A, lane a). *qa-1F* mRNA is transcribed at a low level in uninduced wild-type (*qa-1F*⁺) cultures (lane b). When wild-type cultures are transferred to inducing conditions, *qa-1F* mRNA increases for 4 h, decreases slightly by 8 h, and continues to decrease more noticeably by 10 h (lanes c through g). As indicated, upon induction with quinic acid, a striking 50-fold increase is observed in the level of *qa-1F* mRNA (from 0 to 4 h, lanes c to e). At 10 h (lane g), the *qa-1F* mRNA level is 38% of that at 4 h (lane e). Depletion of the inducer, quinic acid, by the catabolic enzymes encoded by the *qa-2*, *qa-3*, and *qa-4* genes may be related to the decrease in the *qa-1F* mRNA level by 10 h. During induction, the removal of quinic acid causes an immediate decrease in the catabolic enzyme levels (data not shown). We

interpret this observation as an indication that the *qa-1S* repressor protein is activated by the decrease in the level of the inducer quinic acid.

A comparison of *qa-1F* transcription to that of an inducible *qa* structural gene indicates minor differences. For example, *qa-3* mRNA is not detectable in poly(A)⁺ RNA from an uninduced wild-type culture (Fig. 2B, lane a). The *qa-3* gene encodes the enzyme quinate dehydrogenase. Three related *qa-3* mRNAs of 1.6, 1.4, and 1.26 kilobases are present in induced wild-type poly(A)⁺ RNA (Fig. 2B, lane a). These mRNAs are induced approximately 1,500-fold. The level of *qa-3* mRNA follows the same pattern as that of *qa-1F* mRNA after the transfer of wild-type cultures to inducing conditions, in that the level increases to 4 h and then decreases through 10 h (Fig. 2B, lanes c through g).

The conclusion that *qa-1F* regulation is autogenous, i.e., that the *qa-1F* gene controls the synthesis of its own RNA, stems from the observation that *qa-1F* mRNA in poly(A)⁺ RNA from noninducible *qa-1F*⁻ mutant cultures transferred to inducing conditions does not increase. As shown, *qa-1F* mRNA does not increase when a *qa-1F*⁻ mutant culture (M158) is shifted to inducing conditions (Fig. 2A, lane h versus lane i). By contrast, in wild type, the increase is ca. 50-fold (Fig. 2A, lane c versus lane e).

An alternative interpretation of the effects of *qa-1F*⁻ mutations in inhibiting the synthesis of *qa-1F* mRNA is that the *qa-1F* gene may encode a *qa* permease. The initial induced appearance of *qa* enzymes in wild-type cultures is slow, a striking increase only beginning after 2 h (7). This initial lag is also evident in the levels of *qa-1F* and *qa-3* mRNAs. From 0 to 2 h, *qa-1F* mRNA has increased to only

13% of its maximum level at 4 h (Fig. 2A, lanes c through e). *qa-3* mRNA levels follow the same pattern (Fig. 2B, lanes c through e). We explain this lag of ca. 2 h on the requirement for increased synthesis both of *qa-1F* activator protein and, presumably, of a quinic acid permease which controls quinic acid uptake. The existence of an inducible quinic acid permease is supported by the finding that the uptake of radioactive quinic acid (and shikimic acid) is more rapid in constitutive mutants (*qa-1S^c qa-1F⁺*) than in wild type, which takes up inducer at a rate comparable to constitutive mutants only after a considerable lag. The permease is probably under both *qa-1S* and *qa-1F* control, since both *qa-1S⁻* and *qa-1F⁻* mutations block the uptake of quinic acid (H. W. Rines, *Genetics* 74:s230, 1973).

These results make it necessary to consider that any effect of *qa-1F⁻* mutations on transcription of both *qa-1F* mRNA and mRNAs from other *qa* genes might be due to the absence of an active *qa* permease. There are two possibilities. Either the *qa-1F* gene itself encodes a permease or the *qa-1F⁻* mutation produces an inactive activator, therefore blocking transcription of a *qa* permease gene. The first possibility has been excluded by examining *qa-1F* transcription (and *qa-3* transcription) in *qa-1F⁻* mutants and also in *qa-1F⁻ arom-9⁻* double mutants. An *arom-9⁻* mutation in the *arom* cluster gene, which encodes five enzymes in the common biosynthetic aromatic amino acid pathway (10, 13), allows the temporary accumulation of the internal inducer, dehydroquinate. Therefore, the *qa* enzymes are expressed at a low level in a *qa-1F⁺ arom-9⁻* culture grown in the absence of added exogenous inducer (i.e., in sucrose only) (26). Poly(A)⁺ RNA from a *qa-1F⁺ arom-9⁻* culture transferred to sucrose (Fig. 2A, lane j) is calculated to have twice the low level of *qa-1F* mRNA present in an uninduced wild-type culture (Fig. 2A, lanes b and c). There is also a markedly increased level of *qa-3* mRNA (Fig. 2B, lane j; cf. wild-type SUC, lanes b and c). The increased transcription of *qa-1F* mRNA and *qa-3* mRNA is even more striking in *Neurospora* strains, which have mutations that block reactions in the quinate-shikimate catabolic pathway as well as the aromatic amino acid biosynthetic pathway and allow a greater buildup of internal inducer (6). Thus, when a triple mutant (*qa-3⁻ qa-4⁻ arom-1⁻*) culture is transferred to glucose, the *qa-1F* mRNA (Fig. 2A, lane k) is 20 times the low level of an uninduced wild-type culture (i.e., on glucose only; data not shown). The pattern of *qa-3* transcription in this mutant (Fig. 2B, lane k) follows that of *qa-1F* transcription. In contrast to an *arom-9⁻* mutant, *qa-1F⁻ arom-9⁻* double mutants (Fig. 2A, lanes l and m) transferred to sucrose have a low level of *qa-1F* mRNAs, equivalent to that characteristic of uninduced wild-type (Fig. 2A, lane b), and no *qa-3* mRNA (Fig. 2B, lanes l and m). Thus, *qa-1F* mutations prevent *qa* expression in the presence of internal inducer. (Unfortunately, it has not been possible to obtain a *qa-1F⁻* mutation in the triple mutant strain *qa-3⁻ qa-4⁻ arom-1⁻* which accumulates a greater amount of internal inducer than does the *arom-9⁻* strain.)

Thus, it is evident that a mutation in the *qa-1F* regulatory gene influences the expression of both that gene and the inducible *qa* structural genes. Clearly, a wild-type *qa-1F⁺* gene is required for the induced high-level synthesis both of its own mRNA and of the mRNAs of the *qa* structural genes.

Further evidence that the *qa-1F* gene is autoregulated comes from a study of three temperature-sensitive *qa-1F^{ts}* mutants which are inducible for the *qa* enzymes to a limited degree at 25°C and noninducible at 35°C. These mutants produce low basal levels of *qa-1F* mRNA in cultures grown

at either temperature; however, there is a clear increase in *qa-1F* mRNA upon induction with quinic acid at 25°C but little or no increase at 35°C (data not shown).

Control of *qa-1F* transcription by the *qa-1S* gene. Evidence was also obtained that the *qa-1S* gene regulates the synthesis of *qa-1F* mRNA. As shown in Fig. 3 (lanes c and d), a noninducible *qa-1S⁻* mutant (M141), whether transferred to noninducing conditions (sucrose) or inducing conditions (quinic acid), contains a low level of *qa-1F* mRNA essentially equivalent to the low basal level observed in an uninduced wild-type culture (lane a). A similar low level of *qa-1F* mRNA is also observed in a *qa-1S⁻ arom-9⁻* double mutant (Fig. 3A, lanes e and f), ruling out the possibility that the *qa-1S* gene encodes a permease. *qa-3* mRNA is not detectable in the *qa-1S⁻* mutants (Fig. 3B, lanes c through f).

Analysis of a constitutive *qa-1S^c* mutant (M105-R12-1.5) provides additional information on the relationship between the *qa-1S* and *qa-1F* genes. DNA-RNA blot analysis indicates that, in *qa-1S^c* cultures transferred to sucrose (Fig. 3, lane g), *qa-1F* mRNA is 48% of the induced wild-type level. The presence of inducer, quinic acid, does influence *qa-1F* transcription in the *qa-1S^c* strain, since *qa-1F* mRNA in poly(A)⁺ RNA extracted from a culture after transfer from sucrose to inducing conditions (Fig. 3A, lane h) increases to 73% of induced wild-type level. *qa-3* mRNA is present in both cultures (Fig. 3B, lanes g and h).

Further evidence that the *qa-1S* gene also controls expression of the *qa-1F* gene comes from experiments in which the production of *qa-1F* mRNA has been studied in a temperature-sensitive (*qa-1S^{ts}*) mutant. This mutant (M105) is noninducible at 25°C and complements as a *qa-1S⁻* mutant but is constitutive at 35°C. It maps at one end of the *qa-1S* gene adjacent to the *qa-1F* gene (5). The DNA-RNA blot analyses indicate that *qa-1F* mRNA is present in cultures of M105 transferred to quinic acid and to sucrose at both temperatures (Fig. 3A, lanes i through l). However, the level of *qa-1F* mRNA is lowest in cultures grown at 25°C (lanes i and j). In sucrose, it is equivalent to the uninduced wild-type level (lane a), and in quinic acid, a three-fold increase occurs. When mutant cultures are transferred to sucrose at 35°C (lane k), poly(A)⁺ RNA contains four times the *qa-1F* mRNA sequences present in uninduced wild type. The presence of inducer, quinic acid, increases the level of *qa-1F* mRNA in the temperature-sensitive *qa-1S* strain at 35°C (lane l) 14-fold over that of uninduced wild type. However, none of these cultures has the induced level of *qa-1F* mRNA typical of an induced wild-type culture (lane b).

Both catabolic dehydroquinase (*qa-2* gene product) and quinate dehydrogenase (*qa-3* gene product) activities are present in *qa-1S^{ts}* (M105) cultures transferred to sucrose at 35 but not at 25°C. There is an enhancement of both activities in the presence of quinic acid at 35 but not at 25°C. Therefore, these results correlate well with the inducible *qa* structural gene mRNA levels (*qa-3* mRNA results are shown in Fig. 3B, lanes i through l).

DISCUSSION

Prior genetic (5) and molecular (15) data support the hypothesis that the *qa-1F* and *qa-1S* genes play a positive and negative role, respectively, in regulating the expression of the *qa* gene cluster. In this paper, we present evidence supporting a positive role for the *qa-1F* gene in regulating its own expression. Although *qa-1F* mRNA is synthesized at a low level in uninduced wild-type cultures, it is also subject to strong autogenous regulation. The *qa-1F⁺* gene product

FIG. 3. Control of *qa-1F* transcription by the *qa-1S* gene. The *qa-1S* mutant strains used in this experiment were a *qa-1S*⁻ mutant (M141) with and without the *arom-9*⁻ mutation, a temperature-sensitive *qa-1S*^{ts} mutant (M105), and a constitutive *qa-1S*^c mutant (M105-R12-1.5) derived as a "revertant" of M105. Conidia from M141 *arom-9*⁻ were germinated for 48 h and then transferred to noninducing (SUC [lane c]), and to inducing (QA [lane d]) conditions for 4 h. M141 *arom-9*⁻ conidia were grown overnight and transferred to noninducing (SUC [lane e]), and to inducing (QA [lane f]) conditions for 4 h. *qa-1S*^c conidia were germinated for 20 hours and then transferred to noninducing (SUC [lane g]), and to inducing (QA [lane h]) conditions for 6 h. Conidia from the *qa-1S*^{ts} (M105) strain were divided into two portions. The first portion was germinated overnight for 20 hours at 25°C and transferred to noninducing conditions (SUC) and inducing conditions (QA) for 4 hours at 25°C (lanes i and j, respectively); the second portion was also subjected to noninducing and inducing conditions, except at 35°C (lanes k and l, respectively). The RNA blot in panel A was hybridized to pLH40 (cf. Fig. 1B), which indicates the levels of *qa-1F* mRNA. That in panel B was hybridized to pQA3 to detect *qa-3* mRNA (cf. Fig. 1B). Poly(A)⁺ RNA was prepared and electrophoresed as described in Materials and Methods. Conditions for prehybridization, hybridization, and washing of RNA filter are also described in Materials and Methods. The lanes contain the following poly(A)⁺ RNAs: a, uninduced (SUC) wild type, 20 µg; b, induced (QA) wild type, 5 µg; c, uninduced (SUC) *qa-1S*⁻, 20 µg; d, induced (QA) *qa-1S*⁻, 20 µg; e, uninduced (SUC) *qa-1S*⁻ *arom-9*⁻, 15 µg; f, induced (QA) *qa-1S*⁻ *arom-9*⁻, 25 µg; g, uninduced (SUC) *qa-1S*^c, 10 µg; h, induced (QA) *qa-1S*^c, 10 µg; i, uninduced (SUC) *qa-1S*^{ts} at 25°C, 10 µg; j, induced (QA) *qa-1S*^{ts} at 25°C, 10 µg; k, uninduced (SUC) *qa-1S*^{ts} at 35°C, 10 µg; l, induced (QA) *qa-1S*^{ts} at 35°C, 10 µg. Uninduced wild-type and induced (4-h) wild-type poly(A)⁺ RNA (lanes a and b, respectively) are samples from poly(A)⁺ RNA used for Fig. 2.

activates the synthesis of its own mRNA, since *qa-1F* mRNA is present at much higher levels (50-fold) in induced *qa-1F*⁺ wild-type cultures than in cultures of noninducible *qa-1F*⁻ mutants transferred to inducing conditions. The possibility that the effects of *qa-1F*⁻ mutations on *qa-1F* mRNA synthesis arise because the *qa-1F* gene encodes a permease rather than an activator protein is excluded by the analysis of *qa* gene transcription in mutants which accumulate internal inducer.

The results obtained with mutants in the *qa-1S* gene indicate that this gene is also involved in regulating the synthesis of *qa-1F* mRNA. Cultures of a noninducible *qa-1S*⁻ mutant, under inducing conditions, produce a low basal level of *qa-1F* mRNA equivalent to the level present in noninducible *qa-1F*⁻ mutants under comparable conditions and to the levels in *qa-1F*⁺ wild-type cultures under noninducing conditions. Clearly, the induction of the *qa-1F* gene requires wild-type gene products of both the *qa-1S* and *qa-1F* genes.

The results just discussed provide additional evidence for a revised model of regulation in the *qa* gene cluster, based on prior genetic studies of *qa* mutants (5) and recent molecular studies indicating the existence of two distinct regulatory genes in this cluster (15). This model proposes that the two *qa* regulatory genes, designated *qa-1S* and *qa-1F*, encode, respectively, a repressor protein and an activator protein. These two proteins are postulated to act as follows. The *qa-1S*⁺ protein (the repressor) negatively regulates *qa-1F* mRNA expression. *qa-1S*⁻ mutants (semidominant and noninducible) are defective in inducer binding and act as superrepressors, whereas *qa-1S*^c mutants (recessive and constitutive) produce inactive repressors which allow *qa-1F*⁺ expression in the absence of inducer. In wild type, the

repressor interacts with inducer, quinic acid (or related compounds), and permits increased expression of the *qa-1F*⁺ gene. The resulting *qa-1F*⁺ protein then greatly activates the transcription of its own *qa-1F*⁺ mRNA as well as the transcription of the mRNAs for the *qa* structural genes and for *qa-1S*⁺. *qa-1F*⁻ mutants (recessive and noninducible) produce an inactive activator. *qa-1F*⁻ mutants are epistatic to *qa-1S*⁺ and to both *qa-1S*⁻ and *qa-1S*^c mutants, i.e., both *qa-1S*⁺ *qa-1F*⁻ and *qa-1S*^c *qa-1F*⁻ combinations are noninducible (5), indicating that a functional activator protein is absolutely required for induction, regardless of whether or not an active repressor is present. This appears to be the simplest model for the regulatory roles of the *qa-1F* and *qa-1S* genes. Experiments are now under way to determine at what level the *qa-1S* repressor is acting, whether transcriptionally, translationally, or by direct protein interaction with the *qa-1F* activator (12).

The data presented in this paper clearly indicate that the *qa-1F* gene product is involved in activating the synthesis of its own mRNA (and also of the mRNAs of the inducible *qa* structural genes). Most *qa-1F*⁻ mutants are noninducible at both 25 and 35°C and presumably produce either no activator protein or activator molecules which are totally inactive at both temperatures. Such *qa-1F*⁻ mutants also exhibit little or no increase in *qa-1F* mRNA upon induction at either temperature. By contrast, results obtained in the study of three *qa-1F*^{ts} mutants suggest that these *qa-1F*^{ts} mutants are producing temperature-sensitive activator molecules which are partially active at 25°C but inactive at 35°C.

Support for the role of the *qa-1S* product as a repressor comes from our comparative studies of *qa-1S* mutants. A recessive constitutive mutant (*qa-1S*^c) has been shown to produce elevated levels of *qa-1F* mRNA compared with wild

type when both strains are incubated in the absence of quinic acid. Presumably such *qa-1S^c* mutants produce defective repressors which permit increased levels of *qa-1F* expression. By contrast, *qa-1S⁻* mutants are semidominant and noninducible. Presumably, these mutants produce super-repressors which are defective in inducer binding and repress *qa-1F* expression, even in the presence of quinic acid. The one *qa-1S^{ts}* mutant studied also supports a repressor role for a *qa-1S* protein, since it presumably makes a thermolabile repressor which is almost fully active at 25°C but largely inactive at 35°C.

Relatively few genetic regulatory systems have been characterized in any detail at the molecular level in eucaryotes. Of these, the galactose system in *Saccharomyces cerevisiae* appears initially to be most similar to the *qa* system. In this system, two regulatory genes have been cloned, i.e., the *GAL4* gene which exerts positive control (16, 18) and the *GAL80* gene which exerts negative control (21). Douglas and Hawthorne (8) originally proposed that the regulation of three clustered galactose structural genes was due to modulation of *GAL80* repression of *GAL4* synthesis. However, recent evidence suggests that both galactose regulatory proteins are synthesized constitutively and that the role of the *GAL80* protein is to regulate the activity rather than the synthesis of the *GAL4* protein (20, 23). These experiments also provide evidence that the *GAL4* gene has no influence on the synthesis of its own mRNA and is not induced by galactose (18). The level of *GAL4* mRNA in galactose-grown cells has been calculated to be approximately 0.1 mRNA molecule per cell (18). We have estimated the level of *qa-1F* mRNA in induced wild-type cultures to be approximately 0.1 to 1 mRNA molecule per nucleus (see Materials and Methods). These low levels are consistent with the idea that both these genes encode regulatory proteins.

The galactose and *qa* systems appear similar in certain respects, but present evidence suggests that regulation in the two systems differs markedly. The synthesis of *qa-1F* mRNA is clearly under the control, directly or indirectly, of the *qa-1S* gene, the counterpart of the *GAL80* gene. The *qa-1F* gene, the counterpart of the *GAL4* gene, is inducible since the level of *qa-1F* mRNA shifts from low to high upon induction of a wild-type strain by quinic acid. In addition, the data clearly indicate strong, autogenous regulation of the *qa-1F* gene since *qa-1F⁻* (noninducible) mutants make only low levels of *qa-1F* mRNA under inducing conditions. Mutants comparable to the constitutive mutants (*GAL81*) in the *GAL4* gene (20), which indicate an interaction between the *GAL80* and *GAL4* proteins, have not been recovered in the *qa-1F* gene. However, preliminary data obtained in transformation experiments utilizing as donor DNA a *qa-1F⁺* gene that lacks all 5' untranslated sequences as well as part of the transcribed leader sequence (12) suggest that interaction between the two *qa* regulatory proteins may also occur in the *qa* system.

In conclusion, the *qa-1F* regulatory gene apparently encodes an activator protein that positively controls, at the transcriptional level, expression of itself and of the other *qa* genes and is subject to negative control by the *qa-1S* gene.

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